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Combined Effects of Activated Carbon and pH on Ionic Composition
and 2,4-D Availability in a Tissue Culture Medium

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COMBINED EFFECTS OF ACTIVATED CARBON AND pH ON IONIC COMPOSITION AND 2,4-D AVAILABILITY IN A TISSUE CULTURE MEDIUM

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ABSTRACT

Activated carbon (AC) is added to tissue culture medium, often giving positive results. Two different activated carbons have been found to give different success rates in tissue cultures. The two carbons have been characterized using several different techniques with the goal of correlating character with impact on medium composition. Ash %, point of zero charge (PZC), and apparent surface area, showed the greatest differences. Medium pH was found to vary with AC type, and preparation technique. The combination of AC and medium pH (ranging from pH 4.8 to pH 6.8) could significantly alter the ionic composition of the medium, resulting in decreased Cu (-90%), Zn (-50%), Fe (-50%) and Mn (-60%) and increased Mg (+50%). The sorption of 2,4-D varied with apparent surface area (BET) and system pH: the combination of factors resulting in nearly 50% differences in sorption capacity. These findings may help to explain some of the contradiction in the literature regarding the benefits of including AC in tissue culture medium.

INTRODUCTION

The addition of activated carbon (AC) to plant tissue culture medium has been shown to benefit many different tissue culture systems, including algal cultures (1), anther cultures (2, 3), date palm cultures (4), and Douglas-fir and other conifer cultures (5). Several different hypotheses have been advanced to explain the observed benefits: adsorption of growth inhibitors (toxic metabolites) and prevention of unwanted callus growth (6), adsorption of breakdown components of sucrose (7), removal of excess hormone (2,4-D) (8) and release of substance (9) and the sorption of mineral nutrients (10, 11).

Anther culture research has shown that success may vary with the source of activated carbon (12). Work with Douglas-fir somatic embryogenesis at IPST (unpublished results) has revealed that acid-washed activated carbon performs differently from non-acid washed activated

carbon. Further work at IPST using a Norway spruce somatic embryogenesis system has shown that success may also vary with AC production lots.

Research at IPST has shown that the presence of AC in media used for initiation of loblolly pine results in a 50% decrease in available zinc and a 90% decrease in available copper (13). The sorption of 2,4-D onto a single activated carbon was modeled, allowing predictions of available 2,4-D based on initial relative concentrations of 2,4-D and AC (13).

The physical and chemical properties of AC derive from its extensive surface area, which may include a high percentage of micropores (pores less than 2 nm) and its surface chemistry, which is primarily determined by functional groups containing oxygen. Activated carbon is produced through oxidation of pyrolyzed material, which is subsequently pulverized. This oxidation may be achieved with acids, typically nitric or phosphoric, or using high temperature treatments under an oxidizing atmosphere, commonly steam or CO₂. The surface may be either acidic or basic (14).

In this on-going study we seek to correlate the physical/chemical characteristics of activated carbon with the impact of AC on the medium composition, and subsequently, its impact on culture success. This paper reports results for two different activated carbons for the sorption of 2,4-dichlorophenoxy acetic acid (2,4-D) from aqueous solution as well as AC impact on cation concentrations in an initiation medium (Norway spruce).

EXPERIMENTAL

Characterization of Activated Carbon

Activated carbon was supplied by Sigma as untreated powder (C-5260), designated "N" type, and acid-washed tissue culture tested (C-9157), designated "T" type. Two production lots, designated "1" and "2", for each type were characterized. For each characterization, unless stated otherwise, at least two replications were performed.

Ash % was determined on dry AC (dried using a vacuum oven at 120°C overnight) through thermo-gravimetric analysis. The point of zero charge, PZC, was approximated through mass titration (15) which simply involved adding an increasing mass of AC to a given mass of water until some limiting pH value was approached. Apparent surface area (BET) was determined through nitrogen sorption (Micromeritics Flowsorb II 2300).

2,4-D Sorption

Experiments were performed using sealed 40mL glass vials or 200mL plastic bottles. To reach pH targets, acid solution (0.01N or 0.1N HCl) was first added to AC and allowed to equilibrate. The proper amount of acid had been previously determined through titrations and trial and error. Phosphate buffer, adjusted to the final pH target, was added such that the final diluted concentration was approximately 0.0125M. Stock solution of 2,4-D (1g/L) was added to give a final concentration of 200mg/L (200ppm). All aqueous phases were mixed using degassed, deionized water. All vessels were shaken for the duration of the experiment. Experiments were performed in duplicate.

Following sorption, typically longer than five days, samples (10mL) were taken from the vessels and passed through a syringe filter (Gelman Acrodisc 0.2 μ m, HT Tuffryn® membrane). The first 1.5 mL were discarded. The 2,4-D concentration was measured at 284nm (Beckman DU 640 Spectrophotometer).

Cation Analysis

Ion analyses were performed on complete and partial media. Experiments were performed in duplicate. The medium consisted of a liquid version of the Institute of Paper Chemistry Norway spruce initiation medium (16), with 2,4-D (2ppm) substituted for NAA, and BAP (1ppm) as specified previously. When activated carbon was present (1.25 g/L), the hormone levels were elevated to 90ppm BAP and 100ppm 2,4-D. Media were typically allowed to equilibrate three days at room temperature (21°C) before being analyzed. Samples (approximately 7.5mL) were collected as per the 2,4-D procedure. Two drops of concentrated nitric acid were added to each sample. The samples were then analyzed for cations using inductively coupled plasma atomic emission spectroscopy (ICP-AES, Perkin Elmer Optima 3000 DV). Each ICP measurement was replicated three times.

RESULTS

Characterization of AC

Mean values for the characterization results are summarized in Table 1. The ash percentage varied significantly between AC types, with N1 and N2 containing more than twice as much ash percent as T1 or T2. Significant variation in ash % was evident between batches 1 and 2 for the N-type AC. Analysis for trace metals (SEM-EDS) failed to detect significant differences in atomic species present in the ash for the different AC samples: the release of toxic metals does not appear to be a concern when using these AC's.

Table 1. Summary AC Character

	Ash %	PZC, pH	BET m ² /g
T1	2.8	8.5	1050
T2	2.6	10	940
N1	5.8	10.7	560
N2	8.7	11	710

The point of zero charge (PZC) characterization gives the pH at which the sum of the charges on a particle suspension is equal to zero. This measurement differs from the isoelectric point (IEP) in that the isoelectric point is determined from moving particles: the internal surface is not important to IEP determinations. The PZC data indicate that the T1 and T2 are less basic than N1 and N2. Since the pH scale is logarithmic, a pH unit of difference is quite significant at pH 10. It can be concluded that the carbon types are significantly different with respect to PZC. The difference between T1 and T2 indicates that production lots may vary significantly.

The apparent surface area ranged from 560 m²/g to 1050 m²/g. Comparing the average of T1 and T2 to that for N1 and N2, it may be seen that the difference due to carbon type was approximately 350m²/g. The greatest difference was between T1 and N1. These were the two carbons which have been used in tissue culture media at IPST. The between-batch variation, i.e. T1 vs. T2, and N1 vs. N2, appears to be significant.

Culture Medium pH

As a result of the AC characterization, experiments were conducted in which media were mixed, allowed to equilibrate two days and pH measurements made. The two day period was representative of what might occur in actual lab practice.

According to our procedure at IPST, the medium pH was adjusted after adding AC, prior to autoclaving. In this case, the target pH was 5.8. As can be seen from Figure 1, the amount of time allowed for pH adjustment influenced the pH of the medium after two days. Referring to the figure, the x-axis is labeled according to the amount of time allowed to adjust pH after addition of AC to the medium. For the media labeled, "0 min", the pH was adjusted prior to AC addition with no further adjustment after AC addition. As more time was allowed for pH adjustment after AC addition, the closer was the final pH to the target. The tendency, however, was for the pH to drift in the basic direction when AC was present.

After two days, N1 gave higher pH for each treatment, approaching pH 6.8 when no time was allowed for pH adjustment. Past experience has shown that the pH of the

medium just after autoclaving is typically below 5.8, about 5.6 for T-type and above 6.5 for N-type. Titrations with acid have revealed that ten times as much volume is required to reach a given pH point for N-type vs. T-type carbons. Also, it has been found that pH drift after the titration end point has been reached is much more significant for the N-type carbons: it is much more difficult to obtain pH 5.8 with the N-type carbons.

For media exposed to a growing Norway spruce culture, the medium pH drops to about 4.8 over a period of days or weeks. This phenomenon has been observed for media with T-type AC and for media without AC. When N-type carbons have been used, this drop tends to be much more gradual and doesn't dip below pH 5.

Therefore, the pH range of interest for this research is from pH 4.8 to pH 6.8.

Sorption of 2,4-D pH effect.

Data were collected at room temperature (21°C) for sorption of 2,4-D (200mg/L, 200mL volume, phosphate buffer) onto AC (0.125g/L) over the pH range of 4.8 to 6.8. These relative concentrations were chosen after initial experiments revealed that 2,4-D was being depleted at ten to twenty times more than what had been expected based on previous literature (17). It was found that equilibrium was reached within two days but typically more than five days were allowed before measurements were taken. These data have been normalized to sorption capacity at pH 6.8 and are presented in Figure 2. It can be seen that there was a trend for both carbons towards greater sorption of 2,4-D as the pH declined from 6.8. In fact, at pH 4.8 vs. 6.8 the sorption capacity has increased by more than 40% for both carbons.

Apparent surface area.

Using the same relative concentrations of 2,4-D and AC as the previous experiments and adjusting pH to approximately 6.8 for each solution, sorption data were generated for each of the four activated carbons. These data were then plotted as a function of apparent surface area in Figure 3.

The two samples per AC type show a good deal of spread in their sorption capacity, indicating a high level of variability in the material. The mean trend is that sorption capacity for 2,4-D increases with apparent surface area. Comparing mean values for T1 and N1, the difference is about one third more sorption onto T1.

Ion experiments.

Equilibrium conditions were reached within a day but typically two days were allowed before samples were analyzed. Data were initially produced for thirty ions. However, with the exception of silica, neither N1 nor T1 were releasing measurable quantities of non-nutrient ions. The media at pH 4.8 were visibly clearer than media at higher pH. After autoclaving, a precipitate was visible in media with pH above 5.8. The precipitate could be avoided by omitting the macronutrients from the medium.

Figures 4 and 5 present data for copper and zinc, respectively. The concentration of each ion is depicted as a function of medium pH. Media without carbon are designated "Con" short for "control" and were otherwise identical to media which included AC. Across the top of each figure a line is drawn defining the concentration of ion calculated to be present based on the medium "recipe".

From the data in Figure 4 it can be seen that more than 90% of the copper was depleted compared to the control. The control levels were in good agreement with the calculated values. There appeared to be a slight increase in copper availability as pH increased. The data for the two different AC's showed no difference.

Figure 5 shows that the control responded to changes in pH. About 50% of the zinc was depleted in response to a pH increase from pH 5.8 to pH 6.75, without AC present. The presence of AC resulted in reduced availability of zinc compared to the control across the pH range of 4.8 to 6.75. The scatter in the data prevented conclusions as to whether there was an effect due to AC type.

Figure 6 presents data for iron. All of the media were low in iron relative to the calculated amount, across the pH range studied. This phenomenon has been observed previously and attributed to the precipitation of phosphate and Fe-EDTA (18). Relative to the amount of iron present at pH 4.8, about 50% was depleted as pH increased to 6.8. The control sample showed higher precipitation than the activated carbon samples.

Data for magnesium are depicted in Figure 7. Only the media with N1 showed significant deviation from the calculated value: N1 added about 50% more magnesium relative to the calculated value. A pH effect was not observed in the control or for media containing T1.

Manganese showed a very large pH-dependent drop (Figure 8): more than 60% was depleted as pH increased from 5.8 to 6.8. This drop was independent of the presence of AC.

Molybdate was reduced more than 50% across the pH range, independent of AC. Cobalt was reduced to levels near zero and its importance to the medium needs further investigation.

CONCLUSIONS

The T type activated carbons are different physically and chemically from the N-type, with at least 50% more apparent surface area, half as much ash % and a PZC which is 1 pH unit lower than the N-type carbons. There are also differences between the production lots within either type, most notably for BET surface area.

The pH of the medium may have a profound impact on the medium composition. Medium pH is influenced by AC type and how much time is allowed for pH adjustment prior to autoclaving the medium.

The sorption of 2,4-D onto AC appears to be a function of the apparent surface area and the pH of the medium. The combination of effects when comparing media with T1 to media with N1 results in about 40% more 2,4-D sorption capacity for T1 (over 40% more 2,4-D available in media with N-type AC).

Several ions showed increased availability as the pH of the medium declined from 6.8 to 4.8: P (+33%), Mn (+200%), Fe (+140%), Zn (+190%).

Including AC in the medium results in the increased availability of several ions. A greater effect was observed for N1, with increased Si (+30%), Mg (+50%), Ca (+15%), and Al (+300% approx.).

Only two ions appeared to be sorbed onto AC : Cu (-90%) and Zn (-50%). When AC was present, the availability of Cu increased as pH increased.

Figure 9 summarizes a few of the changes in medium composition which would result from substitution of N1 for T1. The data have been normalized against a medium containing T1 at pH 5.8.

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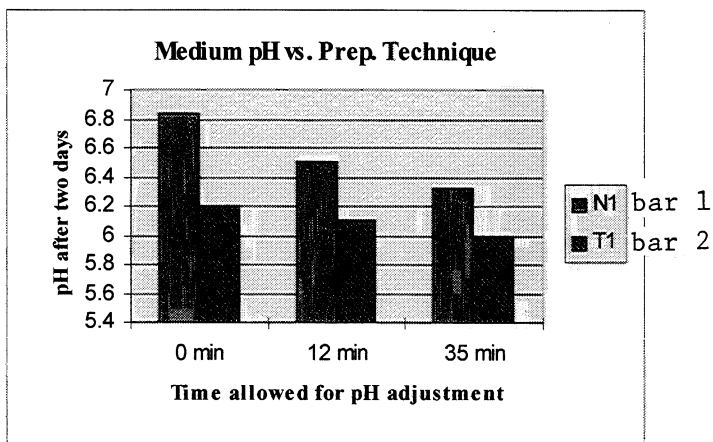


Figure 1. Medium pH as a function of the time allowed for pH adjustment after the addition of activated carbon (1.25 g/L). Media have been adjusted to pH 5.8 and then autoclaved and stored for two days prior to measurement.

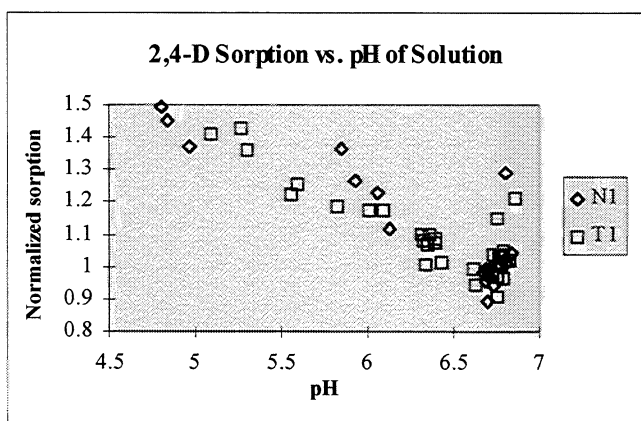


Figure 2. Sorption of 2,4-D (initial conc. 200ppm) onto activated carbon (0.125 g/L) at room temperature from buffered aqueous solution. The data for each carbon type were normalized to their respective mean values at pH 6.8.

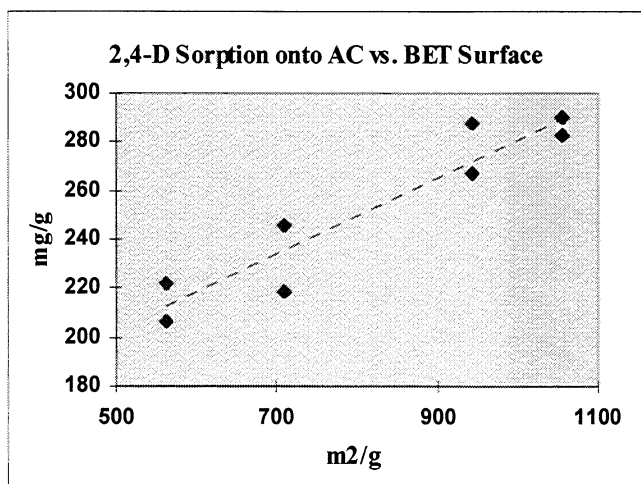


Figure 3. Sorption capacity for 2,4-D as a function of BET surface area of activated carbon. The experimental conditions were: 2,4-D, 200 ppm; AC, 0.125 g/L; pH 6.8; room temp. (21°C). The range in apparent surface area for the four different activated carbons is presented in Table 1.

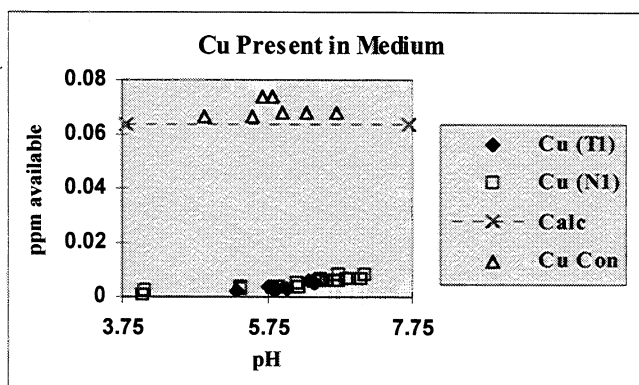


Figure 4. Copper availability vs. medium pH. Data were produced using ICP. Media were complete, including 2,4-D at 100ppm and BAP at 90ppm. Experiments were conducted with one production lot of each AC type, designated T1 and N1. "Calc" designates the amount of ion expected to be present. The control, designated "Con", contained no AC but was otherwise identical to media with AC.

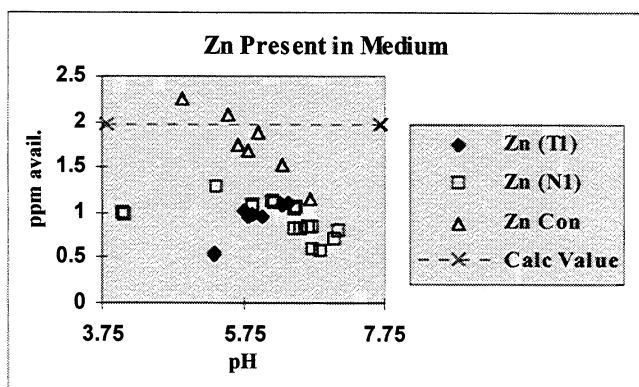


Figure 5. Zinc availability vs. medium pH. See caption for Figure 4.

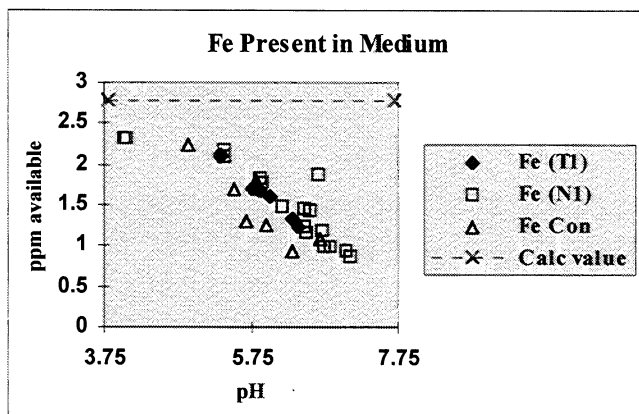


Figure 6. Iron availability vs. medium pH. See caption for Figure 4.

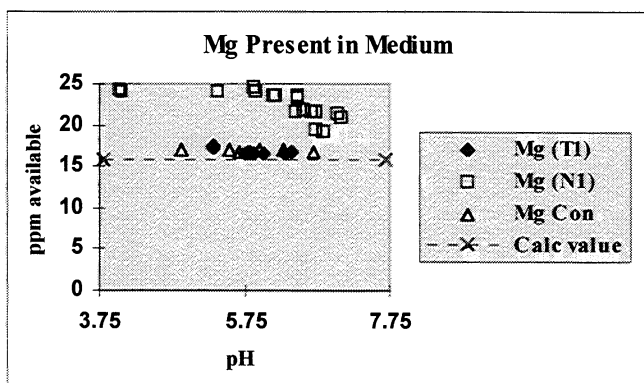


Figure 7. Magnesium availability vs. medium pH. Data were produced using ICP. Media were complete, including 2,4-D at 100ppm and BAP at 90ppm. Experiments were conducted with one production lot for each AC, designated T1 and N1. “Calc” designates the amount of ion expected to be present. The control, designated “Con”, contained no AC but was otherwise identical to media without AC.

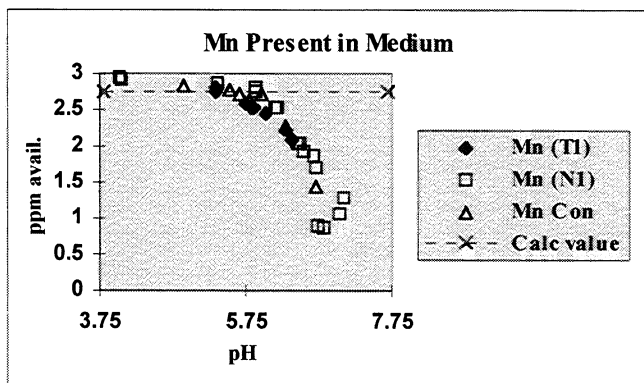


Figure 8. Manganese availability vs. medium pH. See caption for Figure 7.

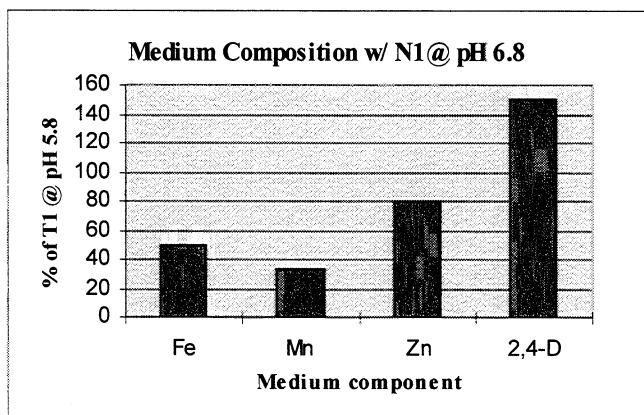


Figure 9. Impact on medium composition of substituting N1 for T1, including pH effects. Data have been normalized to expected medium composition when T1 is present at pH 5.8

